LINC00643 is differentially expressed in the brains of patients with Parkinson's Disease.

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Parkinson's Disease (PD) is characterized by loss of dopaminergic neurons in the substantia nigra of the basal ganglia¹. We mined published and public microarray datasets^{2,3} to identify genes whose expression was most different in the substantia nigra of patients with PD as compared to that of non-affected patients. We identified significant changes in expression of the gene encoding the long intergenic non-coding RNA LINC00643 in the substantia nigra of patients with PD.

Keyword: Parkinson's Disease, motor disorders, substantia nigra, dopaminergic neurons, LINC00643.

1 Parkinson's Disease is a neurodegenerative disorder with no cure. Patients with
2 Parkinson's Disease suffer from motor symptoms including rigidity, tremors, bradykinesia
3 (slowness of movement), and postural instability¹. With disease progression, non-motor
4 neuropsychiatric problems can emerge and worsen, including difficulties with cognition,
5 problems with executive functioning, and problems with mood and behavior¹. 40% of patients
6 with PD develop depression⁴ and approximately 50% of patients with PD will develop psychotic
7 symptoms - hallucinations⁵. Thus, motor and non-motor symptoms are a serious problem in
8 patient with PD. While treatment with dopamine precursor levodopa can provide significant
9 relief, such treatment is marked by "on" and "off" periods of effectiveness and the risk of
10 development of dyskinesias^{6,7}. Newer dopamine agonists such as pramipexole and ropinirole are
11 of limited efficacy⁸ and associated with the risk of impulse control disorders⁹. The development
12 of targeted treatments for patients with Parkinson's Disease, to halt disease progression in the
13 substantia nigra and control motor and neuropsychiatric symptoms in PD patients is required.

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15 We mined published microarray datasets to identify the most striking changes in gene
16 expression in the substantia nigra of patients with Parkinson's Disease. We identified
17 LINC00643 as among the most differentially expressed genes in the brains of patients with PD.
18 LINC00643 may be of relevance to the biology of PD.

25 **Methods**

26 We used datasets GSE42966² and GSE43490³ for this differential gene expression
27 analysis of the substantia nigra in Parkinson's Disease in conjunction with GEO2R. GSE42966
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was generated with Agilent-014850 Whole Human Genome Microarray 4x44K (G4112F) technology with $n=6$ for substantia nigra from control subjects and $n=9$ for substantia nigra from patients with Parkinson's Disease. GSE43490 was generated with Agilent-014850 Whole Human Genome Microarray 4x44K (G4112F) technology with $n=5$ for substantia nigra from control subjects and $n=8$ for substantia nigra from patients with Parkinson's Disease. The Benjamini and Hochberg method of p-value adjustment was used for ranking of differential expression but raw p-values were used for assessment of statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical test was performed to evaluate whether LINC00643 expression was significantly different when comparing substantia nigra from control subjects and from patients with Parkinson's Disease using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses (Version 8.4.0)(455).

Results

We mined published and public microarray datasets^{2,3} to identify differentially expressed genes in patients with Parkinson's Disease.

LINC00643 is differentially expressed in the Parkinson's Disease substantia nigra

We identified the long intergenic non-coding RNA LINC00643 as among the genes whose expression changed most significantly genome-wide when comparing the transcriptional profiles of the substantia nigra from control, non-affected subjects and from patients with

1 Parkinson's Disease (PD)². When sorting each of the transcripts identified by microarray based
2 on degree of change in expression between control subjects and patients with PD, LINC00643
3 ranked 43 out of 45015 total transcripts (Table 1). Differential expression of LINC00643 in the
4 substantia nigra of patients with PD was statistically significant (Table 1; $p=0.004236$).
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6 We mined a second microarray dataset³ to determine if differential expression of
7 LINC00643 in patients with PD could be observed. LINC00643 was again among the genes
8 most differentially expressed when comparing transcriptional profiles of the substantia nigra
9 between patients with PD and control subjects (Table 2). When sorting each of the transcripts
10 identified by microarray based on degree of change in expression between control subjects and
11 patients with PD, LINC00643 ranked 39 out of 22627 total transcripts (Table 2). Differential
12 expression of LINC00643 in the substantia nigra of patients with PD in this second dataset was
13 statistically significant (Table 2; $p=0.0001254$).
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17 LINC00643 is expressed at significantly higher levels in patients with Parkinson's Disease.
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19 We obtained exact mRNA expression levels for the differentially expressed LINC00643
20 transcript, from control subjects and from patients with PD, to directly compare LINC00643
21 expression levels. We also performed a statistical test to determine if this difference in
22 expression was statistically significant. LINC00643 was expressed at higher levels in the
23 substantia nigra of patients with PD, and this difference was statistically significant (Figure 1;
24 $p=0.0061$). We calculated a mean fold change of 1.85 ± 0.81 in LINC00643 expression when
25 comparing substantia nigra from patients with PD to that of control subjects (Table 1).
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1 In the second microarray dataset queried, we again observed increased expression of
2 LINC00643 in the substantia nigra of patients with PD; this difference was statistically
3 significant (Figure 2; $p=0.0120$). We calculated a mean fold change of 1.5399 ± 0.1338 in
4 LINC00643 expression when comparing substantia nigra from patients with PD to that of control
5 subjects (Table 2).
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8 Thus, we identified LINC00643 as a differentially expressed gene in the substantia nigra
9 of patients with Parkinson's Disease and found that LINC00643 was expressed at significantly
10 higher levels in the substantia nigra of patients with PD.
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12 **Discussion**

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14 We mined public and published microarray data to discover genes associated with
15 Parkinson's Disease in the tissues most classically associated with the disease, the substantia
16 nigra of the basal ganglia. We identified the long intergenic non-coding RNA LINC00643 as
17 among the most differentially expressed genes in the substantia nigra of patients with
18 Parkinson's Disease.
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21 RNAs that do not serve as messages, or templates for translation into proteins are known
22 as non-coding RNAs. Non-coding RNAs exist in multiple forms, including long non-coding
23 RNAs (lncRNAs), non-coding RNAs with a length of over 200 basepairs, long intergenic non-
24 coding RNAs, lncRNAs that are located between genes, anti-sense transcripts, intronic
25 transcripts, microRNAs, small nuclear RNAs (snRNAs) and small nucleolar RNAs
26 (snoRNAs)^{10,11}. LINC00643 is a long intergenic non-coding RNA that has been described in the
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1 literature in the context of multiple diseases of the brain.

2 A landmark study that performed RNA-sequencing of brain tissues from 1695 patients
3 with autistic spectrum disorder, schizophrenia and bipolar disorder described LINC00643 as a
4 poorly described, brain-enriched non-coding RNA. LINC00643 expression was observed in
5 GAD1⁺ GABAergic neurons in area 9 of the adult brain, with localization detected in both the
6 cytoplasm and the nucleus as assessed by fluorescence in situ hybridization¹². Expression of
7 LINC00643 in the brain outside of GAD1⁺ GABAergic neurons was also observed. A study
8 identifying long non-coding RNAs in the brain of patients with Alzheimer's Disease found
9 LINC00643 was expressed at significantly lower levels in the Alzheimer's brain when compared
10 to that of age-matched controls¹³. A separate study identifying genes associated with
11 Alzheimer's Disease found significant differential expression of LINC00643. LINC00643 was
12 expressed at significantly lower levels in patients with Alzheimer's Disease, and LINC00643 was
13 also differentially expressed when comparing patient transcriptomes based on age¹⁴. Thus,
14 LINC00643 is expressed in the brain, and in human diseases of the brain, LINC00643 expression
15 is differential or perturbed. Outside of the brain, LINC00643 has been found to be differentially
16 methylated and expressed in the tumors of patients with bladder and gastric cancer^{15,16}. We
17 could not identify any literature describing a role for LINC00643 in patients with Parkinson's
18 Disease.

19 If validated in separate datasets generated with tissues from patients with Parkinson's
20 Disease, the effect of LINC00643 depletion and over-expression *in vivo* in the substantia nigra
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2 can be evaluated through conditional deletion or expression in mice. LINC00643 may be
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4 relevant to the genesis, maintenance or progression of Parkinson's Disease. We found that a
5 brain-enriched, poorly described non-coding RNA was among the genes most differentially
6 expressed in the substantia nigra of patients with Parkinson's Disease.
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Rank	ID	p-value	t	B	FC	Gene	Gene name
43	6310	0.004236	3.288611	-4.46	1.85 ± 0.81	LINC00643	long intergenic non-protein coding RNA 643

Table 1: LINC00643 is differentially expressed in the Parkinson's Disease substantia nigra.

The rank of differential expression, the probe/transcript ID, the *p*-value with respect to global differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the two groups compared, the fold change of LINC00643 in PD substantia nigra when compared to control substantia nigra, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
39	A_24_P840042	0.0001254	4.617033	1.18238	1.5399 ± 0.1338	LINC00643	long intergenic non-protein coding RNA 643

Table 2: LINC00643 is differentially expressed in the Parkinson's Disease substantia nigra.

The rank of differential expression, the probe/transcript ID, the *p*-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, the fold change of LINC00643 in PD substantia nigra when compared to control substantia nigra, the gene and gene name are listed in this chart.

Figure 1: LINC00643 is expressed at significantly higher levels in the substantia nigra of patients with Parkinson's Disease.

The mRNA expression of LINC00643 in normal substantia nigra from control subjects (left) and in the substantia nigra of patients with PD (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in LINC00643 expression between the normal substantia nigra and substantia nigra from patients with PD, a *p*-value, listed above.

Figure 2: LINC00643 is expressed at significantly higher levels in the substantia nigra of patients with Parkinson's Disease.

The mRNA expression of LINC00643 in normal substantia nigra from control subjects (left) and in the substantia nigra of patients with PD (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in LINC00643 expression between control substantia nigra and substantia nigra from patients with PD, a *p*-value, listed above.